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Expression of feline angiotensin converting enzyme 2 and its interaction with SARS-CoV S1 protein

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Abstract

Feline angiotensin converting enzyme 2 (fACE2) gene was amplified from domestic cat lung with RT-PCR, cloned and sequenced. The complete coding region is 2418 bp in length and is the closest to human ACE2 among known ACE2 homologs of non-primate animals. The N terminal fragment 19–367 aa was expressed in *Escherichia coli*. Both Western blotting and ELISA demonstrated that fACE2 could react with SARS-CoV S1 protein as efficiently as ACE2 of Vero E6 cells did.

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Angiotensin converting enzyme 2 (ACE2) was identified as the functional receptor for severe acute respiratory syndrome associated coronavirus (SARS-CoV) in humans (Li et al., 2003; Li et al., 2004; Li et al., 2005a,b). ACE2 is an 805 amino acid carboxy-metalloprotease and plays a significant role in cardiovascular physiology (Eriksson et al., 2002). Several lines of findings demonstrated N terminal of human ACE2 (hACE2), independent of proteolytic site, contains the binding domain of SARS-CoV S1, the receptor binding protein (Hofmann et al., 2004; Li et al., 2003; Yang et al., 2006). Furthermore, the replication limitation of SARS-CoV in non-primate animals is determined by the natural ACE2 homologs from non-primate animals such as mice, rats and palm-civets (Li et al., 2004, 2005a,b). SARS-CoV could infect domestic cats (Martina et al., 2003; Ng, 2003). This study was aimed to clone fACE2 gene from cat tissue and revealed the potential ability of fACE2 to mediate SARS-CoV infection of cats.

Three nine-month-old domestic cats were euthanized. The total RNA of lungs was extracted to amplify fACE2

gene with RT-PCR. Four fragments of fACE2 gene were amplified with the primers based on hACE2 gene (GenBank accession: AF241254) and the resultant fACE2 sequence obtained by the previous steps. The primers for fragment 1 (440–1495 nt in hACE2 gene) are F1: 5'-AGCAAACGGTTGAACAC-3', and R1: 5'-AAGACCATCCACCTCAC-3' specific to hACE2. Fragment 2 (2–831 nt in hACE2 gene) was amplified with primers F2: 5'-GCCCCAACC-CAAGTTCAAAGGCT-3' specific to hACE2 gene and R2: 5'-AAGGGTAGGTATCCATCAAC-3' specific to fragment 1. Fragment 3 from 1287 nt to 2504 nt was obtained with primers F3: 5'-AGATCATGTCACTTTC-TGCG-3' specific to fragment 1 and R3: 5'-ATCAT-CAGTGTTTTTGGGAATC-3' specific to hACE2 gene. Fragment 4 from 2298 nt to the 3' end was amplified using the primer F4 5'-TGATTGTTTTTGGGGTCGT-3' based on fragment 3 and 3' sites adaptor provided by 3' Full Race Core Set (TaKaRa, Dalian, China). As a result, the coding region of fACE2 gene is spliced which is 2418 bp in length, same as that of human (GenBank accession: AF241254), mouse (GenBank accession: AB053181) and rat ACE2 (GenBank accession: AY881244). It encodes 805 amino acids including 17aa of signal peptide sequence and C-terminal transmembrane domain. The similarities to that of

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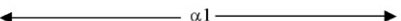


Species	α 1 ridge (aa30-aa41)														Loop and α 3 (aa82-aa93)														Loop and β 5 (aa353-aa357)													
Human	30	D	K	F	N	H	E	A	E	D	L	F	Y	41	82	M	Y	P	L	Q	E	I	Q	N	L	T	V	93	353	K	G	D	F	R	357							
Cat	30	E	K	F	N	H	E	A	E	E	L	S	Y	41	82	T	Y	P	L	A	E	I	H	N	T	T	V	93	353	K	G	D	F	R	357							
Civet	30	E	T	F	N	Y	E	A	Q	E	L	S	Y	41	82	T	Y	P	L	Q	E	I	Q	D	A	K	I	93	353	K	D	D	F	R	357							
Mouse	30	N	N	F	N	Q	E	A	E	D	L	S	Y	41	82	S	F	S	L	Q	E	I	Q	T	P	I	I	93	353	H	G	D	F	R	357							
Rat	30	N	K	F	N	Q	E	A	E	D	L	S	Y	41	82	N	F	S	L	Q	E	I	Q	N	A	T	I	93	353	H	G	D	F	R	357							
																																										

Fig. 1. The comparison of three amino acid regions in ACE2 homologs of different species critical to SARS-CoV S1 association. The single letters represent the amino acid residues and the bold letters represent the different residues from those of human ACE2.

human, mouse and rat ACE2 are, respectively, 85%, 84% and 84% at the nucleotide level and 85%, 81% and 81% at the deduced amino acid level. The completed sequence has been submitted to GenBank (GenBank accession: AY957464).

Three amino acid regions of ACE2 were suggested to be critical to its association to SARS-CoV S protein (Li et al., 2005a,b). The three regions include α 1 ridge (30–41aa), loop and α 3 (82–93aa), loop and β 5 (353–357aa). The sequence of fACE2 in these three regions is closest to that of hACE2 among known sequences of cat, palm-civet, mouse and rat ACE2 (Fig. 1). Meanwhile, among the deduced 18 amino acid residues that contact SARS-CoV receptor binding domain (RBD), fACE2 share the most of them (Li et al., 2005a,b). The number of different amino acids between hACE2 and cat or civet or mouse or rat ACE2 was, respectively, 3, 8, 9, and 11.

The fragment from 19 aa to 367 aa (fACE_{19–367}) excluding signal peptide and proteolytic site was amplified with primers F5: 5'-GATCCATGGGCTCCACCACTGAAGAACTG-3' (the underlined denoted *Nco* I recognition site) and R5 5'-GCATCTCGAGAACAGACACAAAGAATTTC-3' (The underlined denoted *Xho* I recognition site), subcloned into pET28a, and expressed in *Escherichia coli* BL21(DE3) after induction with IPTG 0.08 mmol/L for 3.5 h at 37 °C. The His-tagged fACE_{19–367} was extracted from the inclusion bodies and purified by nickel–nitrilotriacetic acid agarose affinity chromatography (Novagen). SDS-PAGE detected the expression at the size of about 42 kDa as expected. Both fACE_{19–367} and Vero E6 ACE_{219–367} previously expressed by this laboratory (Yang et al., 2006) were separated by SDS-PAGE (12%). Western blot assay was performed with goat polyclonal antibody to hACE2 (R&D) and HRP-coupled goat anti-rabbit IgG (Southern Biotech) (Sambrook et al., 1989). This result showed that fACE_{19–367} could cross-react with the antibody to hACE2. Alternatively, the purified SARS-CoV S1 protein, rabbit antiserum to S1 prepared by this laboratory (Fei et al., 2006) were sequentially overlaid on the blotted membrane. This ligand blotting assay indicated that S1

could detect His-tagged fACE_{19–367} of 42 kDa, as well as GST-ACE_{219–367} of 65 kDa from Vero E6 cells (Fig. 2).

Both fACE2 and Vero E6 ACE2(vACE2) fragments of 1.0 μ g were coated in triplicate in a 96-well plate, and followed by incubation with S1 protein 1.5 μ g/ml. *E. coli* BL21(DE3) transformed with the blank vector was sonicated and taken as the negative control. Finally, the 1000-fold diluted sera of three convalescent SARS patients offered by Hubei Center of Disease Control, 4000-fold diluted HRP-coupled goat polyclonal antibody to human IgG and TMB solution were sequentially added to develop the reaction. The experiment was independently repeated four times. As a result, the mean OD₆₃₀ values for coated vACE2 wells were 0.9094 ± 0.1487 , for coated fACE2 wells were 0.8601 ± 0.1002 , while those of negative control wells were 0.1598 ± 0.0350 . There is no difference in the binding ability of vACE2 and fACE2 to SARS-CoV S1 ($P > 0.01$).

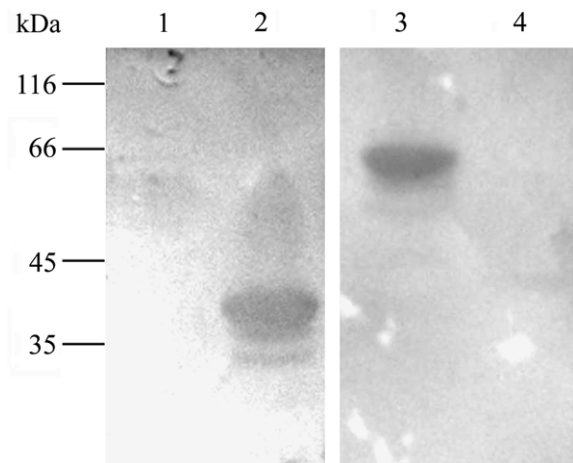


Fig. 2. Binding of ACE2 to SARS-CoV S1. His-tagged fACE_{19–367} and GST-Vero E6 ACE_{219–367} were blotted onto cellulose membrane, probed by SARS-CoV S1 protein, overlaid by anti-S1 antibody. Lanes 1 and 4 represent *E. coli* transformed by blank vector as negative controls. Lanes 2 and 3 represent His-tagged fACE_{19–367} and GST-Vero E6 ACE_{219–367} of 65 kDa. The molecular masses were marked on the left.

However, there is significant difference in OD values between ACE2 and negative control wells ($P < 0.01$).

Taken altogether, we cloned fACE2 gene from domestic cat lung and demonstrated that it possesses the same length of hACE2 and shares a similarity of 85% with that of hACE2 which was confirmed by its cross-reactivity with anti-hACE2 antibody. Among the ACE2 homologs in cats, civets, mice, and rats (Li et al., 2005a,b), fACE2 is evolutionarily closest to hACE2. Both fACE2 and vACE2 could efficiently bind to S1. This would be of significance in zoonotic transmission of SARS-CoV from animals to humans.

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